QuickStix™ Kit for LibertyLink® *(bar)* Cotton Leaf & Seed

Catalog Number AS 013 LS

Highlights:

- *Detects only the PAT/*bar *protein, will not cross-react with the PAT/*pat *protein*
- *Results in 10 minutes or less*
- *Available as 100-strip individual kits, or bulk-packaged strips*

Contents of Kit:

- *100 QuickStix Strips packed in two moisture-resistant canisters*
- *100 Disposable Tissue Extractors, each consisting of a tube with punch cap and pestle (optional item with bulk packaging)*
- *EB2 Extraction Buffer*

Items Not Provided:

- *Seed crushing device: a mallet or pliers can be used to crush individual seeds.*
- *Repeating pipetter or other means of dispensing 0.5 mL*

Contact EnviroLogix to order bulk-packaged kits. Bulk kits include EB2 Extraction Buffer Concentrate. To prepare 1 liter, mix 50 mL 20x Concentrate with 950 mL of distilled or deionized water. Store refrigerated when not in use; allow to come to room temperature before using.

Intended Use

The EnviroLogix QuickStix Kit for LibertyLink® *(bar)* is designed to extract and detect the presence of PAT/*bar* protein at the levels typically expressed in genetically modified cotton seed and/or leaf tissues. It is the only lateral flow strip capable of detecting only the PAT/*bar* protein; it will not cross-react with the PAT/*pat* protein.

How the Test Works

Cotton plants and seeds that have been genetically modified with a Liberty®-tolerant gene express PAT/*bar* protein in their seeds and leaves. To detect the PAT/*bar* protein with the QuickStix Strip, the samples must be extracted and the protein solubilized in buffer provided.

Each QuickStix Strip has an absorbent pad at each end. The protective tape with the arrow indicates the end of the strip to insert into the sample extract. The sample will travel up the membrane strip and be absorbed into the larger pad at the top of the strip. The portion of the strip between the protective tape and the absorbent pad at the top of the strip is used to view the reactions as described under “Interpreting the Results”.

Sample Preparation

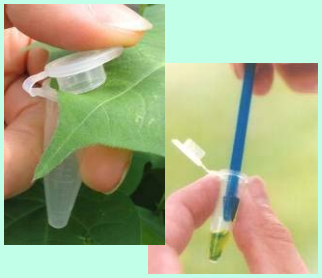
Note: *If Extraction Buffer has been refrigerated, allow it to warm up to room temperature before preparing samples.*

To extract cotton leaf tissue:

1. Sandwich a section of leaf tissue between the cap and body of a Disposable Tissue Extractor tube; snap one or two circular tissue punches by closing the cap. Push the leaf punches down into the tapered bottom of the tube with the pestle. Sample identification should be marked on the tube with a waterproof marker.
2. Insert the pestle into the tube and grind the tissue by rotating the pestle against the sides of the tube with twisting motions. Continue this process for 20 to 30 seconds, or until the leaf tissue is well ground.
3. Add 0.5 mL Extraction Buffer.
4. Repeat the grinding step to mix tissue with Extraction Buffer. Dispose of the pestle (do not re-use pestles on more than one sample).

To extract cotton seed tissue:

1. Crush a single cotton seed (*Suggestion: Use pliers with seed in resealable bag).* Transfer to a Disposable Tissue Extractor Tube marked with sample identification. Seeds may also be crushed with pliers directly in the Extractor Tubes, or mechanically crushed using equivalent methods.
2. Carefully dispense 0.5 mL of Extraction Buffer into the tube containing cotton seed tissue.
3. Close the tube cap securely and shake the tube vigorously for 20 to 30 seconds. Allow the solid material to settle to the bottom of the tube.



Obtain Leaf Tissue, grind

~or~

Crush single seed, add Buffer

Insert QuickStix

Any clearly discernable pink Test Line is considered positive

4. Repeat the protocol for each sample to be tested, using a new tube for each. Use caution to prevent sample-to-sample cross-contamination with plant tissue, fluids, or disposables.
5. Use crushed seed samples the same day they are prepared.

To improve extraction efficiency:

- Use room temperature to lukewarm buffer to extract the seeds.
- Longer soak times can increase the strength of the Test Line (better extraction).
- The extract takes on a yellow to brown opaque color when the seeds are crushed and mixed properly. If the extract is clear, the seed coat may be empty or the sample may not be well mixed. The seeds should contain an adequate amount of mature endosperm and embryonic tissues, not empty seed coats.

How to Run the QuickStix Test

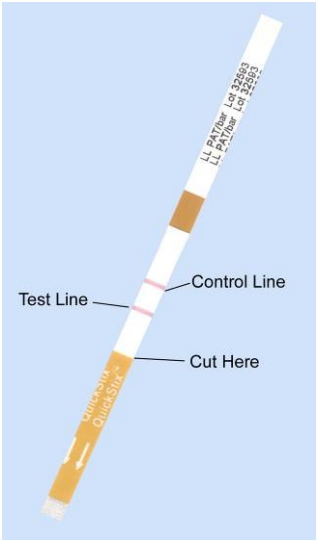
1. Allow refrigerated canisters to come to room temperature before opening. Remove the QuickStix Strips to be used. Avoid bending the strips. Reseal the canister immediately.
2. Place the strip into the extraction tube. The sample will travel up the strip. Use a rack to support multiple tubes if needed.
3. Allow the strip to develop for 10 minutes before making final assay interpretations. Positive sample results may become obvious much more quickly.
4. To retain the strip, cut off and discard the bottom section of the strip covered by the arrow tape.

Interpreting the Results

Development of the Control Line within 10 minutes indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded and the sample re-tested using another strip.

If the sample extract contains the PAT/*bar* protein, a second line (Test Line) will develop on the membrane strip between the Control Line and the protective arrow tape. The results should be interpreted as positive for PAT/*bar* protein expression.

If no Test Line is observed after 10 minutes, the results should be interpreted as negative. A negative result means the sample contains less PAT/*bar* than is typically expressed in the tissues of modified cotton plants.



Kit Storage

This Kit can be stored at room temperature, or refrigerated for a longer shelf life. Please note the shelf life on the kit label for each storage temperature. The kit may be used in field applications; however, prolonged exposure to high temperatures may adversely affect the test results. Do not open the desiccated canister until ready to use the test strips.

Precautions and Limitations

- This kit is designed for screening for presence or absence only and is not meant to be quantitative.
- As with all tests, it is recommended that results be confirmed with an alternate method if necessary.
- The assay has been optimized using the protocol and buffer provided in the kit. Deviation from this protocol may invalidate the results of the test.
- The results generated through the proper use of this kit reflect the condition of the working sample directly tested. Extrapolation as to the condition of the originating lot from which the working sample was derived should be based on sound sampling procedures and statistical calculations which address random sampling effects, non-random seed lot sampling effects, and assay system uncertainty. A negative result obtained when properly testing the working sample does not necessarily mean the originating lot is entirely negative for the analyte or protein in question.
- A negative result with this kit does not mean that the sampled tissue has not been otherwise genetically modified.
- Warning: a strong positive result may safely be interpreted in as little as 2 minutes after sample addition. It is not safe, however, to conclude that a sample is negative before a full 10 minutes has elapsed, as a weak positive sample may require the full 10 minutes for a distinct Test Line to appear.
- Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in vehicle.

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